# Effects of dietary zinc depletion on seminal volume and zinc loss, serum testosterone concentrations, and sperm morphology in young men[1–4]

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**ABSTRACT** Identification of the andrological variables most sensitive to zinc depletion would expedite the diagnosis of male reproductive pathology induced by zinc deficiency. Eleven volunteers living on a metabolic ward were fed a diet composed of a mixture of a semisynthetic formula and conventional foods supplemented with $ZnSO_4$ to supply a total of 1.4, 2.5, 3.4, 4.4, or 10.4 mg Zn/d. After an equilibration period of 28 d (10.4 mg Zn/d), all treatments were presented for 35 d each, the first four in random order and the fifth last. Compared with when they were consuming 10.4 mg Zn/d, volunteers consuming 1.4 mg Zn/d exhibited decreased semen volumes (3.30 vs 2.24 mL) and serum testosterone concentrations (26.9 vs 21.9 nmol/L), and no change in seminal zinc concentrations. Compared with 10.4 mg Zn/d, treatments of 1.4, 2.5, and 3.4 mg Zn/d decreased the total semen zinc loss per ejaculate (6.29 vs 3.81, 4.68, and 5.03 μmol/ejaculate). Seminal loss accounted for 9% of total body zinc loss when 1.4 mg Zn/d was consumed. Seminal phosphorus concentrations were elevated during all four phases of zinc depletion (28.4 vs 32.9, 31.0, 34.2, and 33.6 mmol/L). The findings suggest that serum testosterone concentrations, seminal volume, and total seminal zinc loss per ejaculate are sensitive to short-term zinc depletion in young men. *Am J Clin Nutr* 1992;56: 148–57.

**KEY WORDS** Zinc, semen, testosterone, sperm, human, semen volume, ejaculation frequency, sperm density, calcium, magnesium, iron, phosphorus, potassium, sodium

## Introduction

Understanding human male infertility induced by dietary zinc deficiency is complex because zinc is thought to be involved in several integrated processes associated with reproduction.

Hypogonadism and lack of secondary sexual characteristics have been reported in several severely undernourished young men. These abnormalities responded quickly to dietary supplemental zinc (1). Clinical studies with adult males experimentally deprived of zinc show that Leydig cell synthesis of testosterone is dependent on adequate dietary zinc (1). There is also some evidence that zinc is required for normal functioning of the hypothalamic-pituitary-gonadal axis (2). On the other hand, there is considerable evidence that zinc deficiency causes primary testicular failure and altered testicular steroidogenesis (3).

Zinc is required for the $G_1$ (4) and S (5) phases of the cell cycle. Perhaps more importantly, zinc seems to have a role in the processes required to alter the genetic expression of a cell between the $G_1$ and S, the S and $G_2$, and the $G_2$ and M phases of the cell cycle (6–8).

Several studies have shown that oral zinc supplementation improves sperm motility in subfertile men with idiopathic asthenozoospermia and/or oligozoospermia (9). However, the results of one study indicated that high semen zinc concentrations were related to depressed sperm motility in ejaculates of infertile men (10). Also, motility is apparently unrelated to zinc concentrations in spermatozoa (11).

Seminal zinc is considered an index of prostatic function (12) although the function of zinc in seminal plasma and semen is unknown (13). Most secreted prostatic zinc in humans seems to target the secreted seminal vesicle proteins (14). Other research indicates that the zinc and albumin secreted from the prostate form a complex that coats the sperm and thereby protects the cells (15). Furthermore, prostatic zinc may have antibacterial activity because *Trichomonas vaginalis* is readily killed at concentrations of zinc that occur in the prostatic fluid of healthy men (16).

Because of the critical role of zinc in male reproductive potential, it is important to identify the andrological variables most sensitive to zinc depletion. Assessment of those variables in clinical cases of possible zinc deficiency would expedite treatment. An andrological substudy was therefore incorporated into a human experiment designed to assess zinc homeostasis during short-term severe zinc depletion.

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[2] Presented in part at the Seventh International Symposium on Trace Elements in Man and Animals, Dubrovnik, Croatia, Yugoslavia, May 20, 1990 and at the 1990 North Dakota Academy of Science Meeting held in Fargo, ND, April 19, 1990.

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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## Subjects and methods

### *Experimental design*

Eleven men (28.1 ± 5.0 y of age, $\bar{x}$ ± SD) were selected to live on a metabolic ward for 208 d. Initial body weight was maintained (±2%) by prescription exercise and adjustments in energy intake. Subjects were fed a basal diet (described below) supplemented with $ZnSO_4$ to supply a mean total of 1.44, 2.45, 3.37, 4.43, or 10.35 mg Zn/d. After an equilibration period of 28 d (10.4 mg Zn/d), all treatments were presented for 35 d each, the first four in random order and the 10.35 mg/d treatment last. All dietary treatments except the equilibration period and the last period were double-blinded. Diets were identical during all dietary treatment periods except for the zinc supplement, which was provided as $ZnSO_4$.

For stable-isotope studies (to be reported separately), subjects were infused intravenously with 4 mg stable $^{67}Zn$ 10 d before the end of each dietary period. The following day they were given an oral dose of 1 mg $^{70}Zn$. On days when stable isotopes were administered, other zinc supplements above the basal dietary amount were eliminated or reduced in proportion to the amount of stable zinc administered to perturb daily zinc intake as little as possible.

Under the conditions described below, each volunteer provided semen samples at the beginning, middle, and end of each treatment period (approximately every 2 wk) between 0630 and 0800. The volunteers were divided arbitrarily into two groups, and samples were collected from both groups 1 to 2 d apart. In addition, the frequency of voluntary and involuntary ejaculations was recorded daily by each volunteer on a confidential form that was collected daily. Smoking, alcohol consumption, and drug use were prohibited and random screening was done to monitor compliance.

### *Volunteer selection*

Volunteers were selected on the basis of medical, psychological, and nutritional data obtained from physical examinations and written and oral questionnaires. Data from a separate written andrology questionnaire were used to screen applicants for impaired andrological function caused by anatomical malformation, vasectomy, varicocele, surgery, mumps, urinary infections, venereal disease, hernia, bilateral cryptorchidism, or inadequate libido or virilization (12). After selection, each volunteer signed a consent statement after receiving both oral and written presentations of the nature of the research. The study protocol was approved by the Institutional Review Board of the University of North Dakota and the Human Studies Committee of the US Department of Agriculture. The protocol followed the guidelines of the Department of Health and Human Services and the Helsinki Doctrine regarding the use of human subjects. All subjects were chaperoned when they left the metabolic unit to prevent ingestion of unauthorized foods.

### *Diet*

The basal diet supplied an average of 1.44 mg Zn/d and was composed of conventional foods (**Table 1**) and an additional protein source provided as a semisynthetic formula based on egg white protein (**Table 2**). Supplemental zinc was provided as $ZnSO_4$ (JT Baker, Philipsburg, NJ) added to the formula.

The diet was planned as a weighed metabolic diet on a 3-d rotating menu cycle providing 9.4–13.5 MJ energy/d (2250–3250 kcal/d) at 1.0-MJ (250-kcal) intervals. The 10.5-MJ diet was the baseline from which other energy levels were derived. To minimize dietary sources of zinc, the diet consisted primarily of fruits, juices, vegetables, low-protein starch products, refined-carbohydrate desserts, and a high-protein beverage prepared from the egg white. Grains and animal products were used sparingly; chicken breast was the only meat served, and portion sizes for chicken ranged from 27 to 39 g. Nondairy creamer and whipped topping were used as milk substitutes, and low-protein bread and gluten-free pasta replaced traditional breads and noodles. In addition to the basal diet, volunteers were allowed to consume limited quantities of several low energy foods containing no significant amount of zinc. Salt, pepper, and coffee were served in constant amounts, selected by each volunteer, throughout the study. Up to 0.95 L Diet 7-Up (Coca-Cola West, Bismark, ND) and four sticks of sugarless gum were allowed per day. All other ingredients of the diet were weighed to within 1% accuracy.

The high-protein fruit drink was served three times a day, once each at breakfast, lunch, and supper, with three flavors rotated daily. It was prepared in bulk, with all ingredients weighed individually into stainless-steel blenders and processed until homogenous; specific amounts were weighed into glass tumblers that were covered and frozen until the day of service. Drinks were served thawed or semithawed. Three servings of the fruit drink contained ≈3.46 MJ (826 kcal) (33%), 152 g carbohydrate (39%), 31 g protein (53%), 11 g fat (15%), and 0.22 mg Zn (16%) at 10.5 MJ.

The limited menu was supplemented with some nutrients (**Table 3**): biotin, 300 μg/d (Freeda Vitamins, Inc, New York); B-complex vitamin, containing 0.7 mg thiamin, 0.7 mg riboflavin, 9.0 mg niacin, 0.9 mg vitamin B-6, and 2.0 μg vitamin B-12 (ER Squibb and Sons, Inc, Princeton, NJ); calcium, 270 mg/d as three 1-g calcium gluconate tablets providing 90 mg elemental Ca each (Eli Lilly and Co, Indianapolis); copper, 1.3 mg/d as $CuSO_4$ (JT Baker Chemical Co, Glen Ellyn, IL); iron, 30 mg/d as Fergon brand ferrous gluconate elixir, dispensed into beverages in 1.4-g doses of ferrous gluconate (providing 10 mg Fe/dose) at breakfast, lunch, and supper; magnesium, 85.5 mg/d as three 500-mg tablets of magnesium gluconate (Towne) served separately at breakfast, lunch, and supper; and potassium, 314 mg once every 3 d as 0.30 g KCl (JT Baker, USP grade) added to the lunch casserole and to the chicken at dinner on day three.

### *Semen collection*

There is no standardized procedure reported in the literature for semen collection for trace element research. Thus, detailed semen collection procedures are described below. Semen samples obtained from staff members were used to determine the amount of sample required for all assays and processing times.

The most probable sources of mineral contamination of the semen specimens were mineral leaching from glass containers, desquamitized epithelia, hair, and unapproved lubricants. Therefore, a nonlubricated, nonspermicidal condom over the penis was chosen as the most appropriate method for semen collection. Mineral integrity of the sample was preserved at the risk of reducing sperm motility, a phenomenon observed to occur during even brief contact of sperm with latex.

To select a condom brand, six condoms from a given brand were unrolled inside out and placed in a 14-mL, acid-washed polypropylene tube (Becton-Dickinson Labware, Lincoln Park,

TABLE 1
Three-day rotating menu

| | Day 1 | Day 2 | Day 3 |
|---|---|---|---|
| Breakfast | Cranapple juice | Orange juice | Apple juice |
| | Cornflakes* | Corn Chex† | Trix‡ |
| | Nondairy creamer | Nondairy creamer | Nondairy creamer |
| | Peaches | Blueberries | Cherries |
| | Orange fruit drink | Lemon fruit drink | Orange fruit drink |
| Lunch | Orange juice | Apple juice | Grape juice |
| | Chicken-vegetable casserole | Barbecued chicken | Chicken-potato bake |
| | Rusks | Anellini noodles, buttered‡ | Rusks |
| | Butter | Steamed cauliflower | Butter |
| | Cherry Jello with pineapple§ | Strawberry/banana Jello§ | Apple crisp |
| | Lemon fruit drink | Whipped topping | Lemon fruit drink |
| | | Tropical fruit drink | |
| Dinner | Apple juice | Pineapple juice | Orange juice |
| | Crispy chicken | Chicken-vegetable soup | Baked chicken |
| | Ditalini noodles, buttered‡ | Lettuce salad | White bun |
| | Lettuce salad | French dressing | Lettuce salad |
| | Russian dressing | Rusks | French dressing |
| | Shortbread cookies | Butter | Angelfood cake |
| | Tropical fruit drink | Cherry crisp | Tropical fruit drink |
| | | Orange fruit drink | |
| Snack | Lemonade | Vanilla wafers | Lime Jello with pears§ |
| | Angel food cake | Applesauce | Whipped topping |
| | Whipped topping | | |

* Kelloggs, Battle Creek, MI.
† Ralston Purina Co, St Louis.
‡ Plada, Milan, Italy.
§ General Food Corp, White Plains, NY.

NJ) that contained 4-mL of phosphate buffer. The 0.0038-mol/L $KH_2PO_4$ buffer was selected to partially simulate semen mineral content and pH (7.4). After 24 h the undiluted buffer was analyzed for mineral content by inductively coupled argon plasma spectroscopy (ICP/5000; Perkin-Elmer, Norwalk, CT) as described in detail elsewhere (18). The phosphate buffer served as the blank.

On the basis of the mineral analyses, condoms from one lot of a single brand (Trojans rolled latex condoms, number 75, Y13069-03; distributed by Carter Products, Division of Carter-Wallace, Inc, New York) were selected. On receipt of the selected condoms, one condom in each package of 12 was opened and tested for mineral residue as described above. All buffer aliquots exposed to the selected condoms contained minerals in concentrations below detection limits. Liquid handsoap (Softsoap, Softsoap Enterprises, Inc, Chaska, MN) and paper toweling (Kimwipes, Kimberly-Clark, Corp, Roswell, GA) were selected on the basis of mineral content.

Each volunteer was provided with a styrofoam box that contained a plastic bottle of liquid handsoap; a squeeze-bottle of water (deionized, 18 M $\Omega$ $cm^{-1}$; Millipore System, Super-Q, Millipore Corp, Bedford, MA); paper toweling; two packaged, nonlubricated latex condoms; a 30-mL widemouth polypropylene bottle; a stopwatch; and a questionnaire. The box and all contents were marked with the unique identification number assigned to each volunteer.

The integrity of the semen sample, up until the time of transport to the clinical laboratory, was dependent on the trustworthiness and diligence of the volunteer. Thus, great effort was expended to ensure a high degree of compliance. For instance, the andrology reporting protocol was designed to minimize false reports stemming from psychological factors. In addition, all volunteers were thoroughly briefed on the goal of the andrology research and the concept of microcontamination.

Each volunteer was given detailed oral instructions in both group and individual settings and a written guide for the semen collection protocol. The volunteers were asked to refrain from

TABLE 2
Composition of the high-protein fruit drink*

| Food | Amount |
|---|---|
| | *g/serving* |
| Tang, orange flavored, powder† | 45.00 |
| Lemonade powdered, sweetened | 45.00 |
| Tropical Punch, Kool-Aid, sweetened powder† | 45.00 |
| Water, deionized | 60.00 |
| Egg white, frozen, thawed | 100.00 |
| Nondairy creamer, unsaturated fatty acid, frozen thawed | 35.00 |

* For the 10.5-MJ energy intake. Subjects received lemonade, punch, or Kool-Aid.
† Kraft General Food Inc, White Plains, NY.

TABLE 3
Composition of the diet for the 10.5-MJ energy intake*

| | |
|---|---|
| Calculated values | |
| Energy (MJ) | 10.5 |
| Protein | |
| (g) | 58 [63] |
| (% of energy) | 9 |
| Carbohydrate | |
| (g) | 393 |
| (% of energy) | 64 |
| Crude fiber, (g) | 204 |
| Fat | |
| (g) | 74 |
| (% of energy) | 27 |
| Linoleic acid (g) | 20.1 |
| Total saturated fatty acids (g) | 22.8 |
| P:S† | 0.89 |
| Cholesterol (mg) | 86 |
| Vitamin A (RE) | 1632 [1000] |
| Thiamin (mg)‡ | 1.52 [1.5] |
| Riboflavin (mg)‡ | 2.29 [1.7] |
| Niacin (mg)‡ | 23.1 [19] |
| Ascorbic acid (mg) | 420 [60] |
| Vitamin B-6 (mg)‡ | 2.07 [2.0] |
| Vitamin B-12 (μg)‡ | 3.22 [2.0] |
| Folacin (μg) | 449 [200] |
| Vitamin D (μg)‡ | 10 [5] |
| α-Tocopherol (mg) | 4.27 [10] |
| Biotin (μg)‡ | 300 |
| Analyzed values | |
| Potassium (mg)‡ | 2077 [2000]§ |
| Phosphorus (mg) | 570 [800] |
| Calcium (mg)‡ | 514 [800] |
| Iron (mg)‡ | 40 [10] |
| Magnesium (mg)‡ | 218 [350] |
| Zinc (mg)‖ | 1.36 [15] |
| Copper (mg)‡ | 1.80 [1.5–3.0]¶ |
| Manganese (mg) | 1.32 [2.0–5.0]¶ |
| Sodium (mg)‡ | 1949 [500]‖ |

* Recommended dietary allowances (17) for males aged 25–50 y in brackets.
† Ratio of polyunsaturated to saturated fatty acids.
‡ Includes supplement.
§ Estimated minimum requirement for healthy persons (17).
‖ Without supplement.
¶ Estimated safe and adequate intake (17).

voluntary ejaculation 48 h before semen collection. Hands were washed (tap water), rinsed, and dried with the materials provided. The penis only was cleaned with the materials provided in the andrology kit. Uncircumcised individuals were encouraged to take special cleaning precautions. The condom was removed from the packaging, inspected for imperfections, and rolled completely onto the erect penis. A second condom was provided in case of imperfection or contamination. The collection was to be achieved without any lubricating substance.

After a single ejaculation, the condom with ejaculate was placed in the widemouth bottle in an upright position with the top of the condom folded back around the outside of the bottle. The lid was screwed onto the bottle with great care being taken so that no portion of the ejaculate was either lost or contaminated. The stopwatch was then turned on and the technical staff was notified by telephone to retrieve the entire andrology kit from the two-way refrigerator in the bathroom. The volunteer was also instructed to fill out a seven-item confidential Andrology Data Card, which aided the technical staff in assessing the integrity of the specimen, and/or the need to reschedule collection and indicated the number of hours since the last voluntary ejaculation. Each andrology kit was transported from the metabolic ward to the clinical laboratory in < 2 min by the technical staff. Immediately on receipt, the tip of the condom was cut and the ejaculate was expressed into a graduated polypropylene tube for the determination of ejaculate volume. Elapsed time was recorded for specimen receipt, condom rupture, and every phase of histological, biochemical, and mineral preparation described below.

*Semen sample processing*

Within 5 min of elapsed time and within 1 min after condom rupture, all samples were inspected visually for clots and gross integrity. Then aliquots for all the following assays were removed with plastic pipette tips in the following order: *1*) A semen smear (20 μL) was transferred to a glass slide coated with dried hairspray. The smear was air-dried; dip-stained with triarylmethane, xanthene, and thiazine (S/P Diff-Quik Differential Staining set, Scientific Products, Minneapolis) to give staining characteristics similar to a Wright-Giemsa stain; and covered with a coverslip. *2*) A 20-μL sample to assay sperm motility (% vigorously swimming at 5 and 30 min postcollection) was transferred to a glass slide that was covered with a coverslip and assessed by visual analysis with a microscope. *3*) A 100-μL sample was taken for sperm angiotensin converting enzyme activity (ACE) determination. and *4*) A sample was taken for histological analysis (described below). After allowing the remaining specimen to liquefy at room temperature for 30 min, the sample was mixed gently with a vortex mixer (Scientific Products) and aliquots for the following assays were removed with plastic pipette tips in the following order: *1*) 1000 μL for mineral analysis was transferred to a polypropylene 1500-μL microcentrifuge tube with screwcap (Lab Source, Chicago) and stored at −80°C; *2*) 100 μL for acrosin activity was transferred to a 5-mL polypropylene tube and prepared as described below; *3*) 100 μL was used for histological analysis (described below); *4*) 200 μL was used for determination of secreted protein that is acidic and rich in cystine (SPARC); *5*) 20 μL was used for semen smear, prepared as above; and *6*) < 10 μL for sperm-density measurements was transferred to a glass white blood cell diluting pipet (Scientific Products). The 1:20 dilution with sperm diluent solution (1 mL 37% formaldehyde; 5 g sodium bicarbonate; 100 mL distilled, deionized water) was rocked for 3 min in a pipet shaker, then transferred to a hemocytometer (Spencer, Bright Line, improved Neubauer, $^1/_{10}$ mm deep, Reichert-Jung, Cambridge Instruments, Inc, Buffalo, NY) for counting (19). The sperm-density procedure was done last to avoid specimen contact with glass. Data from the ACE and SPARC assays will be reported later. When the total amount of specimen volume was inadequate for all individual assays, the following priority protocol was observed: mineral analysis, sperm density, motility, 5-min smear, 30-min smear, 0-min microscopy, 30-min microscopy, ACE, acrosin, and SPARC.

*Biochemical analysis*

On the same day as collection, each aliquot for acrosin analysis was layered on 11% Ficoll (F-9378; Sigma Diagnostics, St Louis)

in a 5-mL polypropylene tube. The sample was then centrifuged at 12000 × *g* for 2 min, and the sperm pellet was recovered, washed in 0.9% NaCl without resuspension, and subsequently stored at −80°C. At the time of analysis, the pellet was thawed at room temperature and subsequently assessed for acrosin activity by established procedures (20, 21).

On the last day in each 35-d dietary period, blood was drawn by standard phlebotomy techniques between 0600 and 0700 after 10 h of fasting, for determination of serum total unconjugated testosterone (radioimmunoassay; Radioassay System Laboratories, Carson, CA) and plasma zinc concentrations (analysis described below).

*Histological analysis*

The semen smears collected at 0 and 30 min were analyzed for sperm head, acrosome, and postacrosomal areas; sperm-head aspect ratio (length/width), sperm-head convex perimeter [perimeter of the irregular polygon circumscribing a feature formed by the tangents to its boundary; $2 \times (\text{Tan } (\pi/2n)) \times \sum$Feret diameters measured, where $n$ is the number of measured Ferets, which is the maximum dimension in a specified direction; likened to the length of a piece of string pulled tight around the feature], and sperm-head roundness (convex perimeter/perimeter). All measurements were obtained by image-analysis methods (22) with a model 970 Quantimet Image Analysis System with Chalnicon scanner (Cambridge Instruments, Inc, Monsey, NY). The acrosome was defined as the lightly stained anterior portion of the spermatozoa head. The postacrosomal area was defined as the portion of the head between the posterior border of the acrosome and anterior to the insertion of the flagellar midpiece with the head.

*Mineral analysis*

The 1000-μL aliquots for mineral analysis were allowed to thaw at room temperature. Subsequently, the aliquots were mixed gently with a vortex mixer, then two 500-μL subaliquots from each aliquot were wet-ashed and assayed for calcium, iron, magnesium, phosphorus, potassium, sodium, and zinc concentrations by the method of Hunt and Shuler (18). All subaliquots for a single volunteer were analyzed as one batch. Plasma zinc was determined by flame atomic-absorption spectrometry (AAS) as previously described (23).

*Statistical analysis*

The motility- and ejaculation-frequency data were analyzed nonparametrically by the Friedman test (BMDP, BMDP Statistical Software, Inc, Los Angeles). Mineral concentration, acrosin activity, sperm acrosome area, sperm-head area, sperm-head aspect ratio, and sperm density were analyzed by repeated-measures analysis of variance (ANOVA) followed by Dunnett's comparisons (SAS/STAT; SAS Institute, Inc, Cary, NC) between each zinc-deficient dietary period (1.4, 2.5, 3.4, or 4.4 mg Zn/d) and the zinc-adequate dietary period (10.4 mg Zn/d). Only data collected at the end of each dietary period are described here.

## Results

*Evidence of sample integrity and compliance*

For each volunteer, there was no indication that the mineral integrity of any of the samples was compromised as calculated indirectly (no consistency in percent magnitude or direction of fluctuations in seminal mineral concentrations among elements or among adjacent collections). The absence of motile sperm in samples purported to be collected within 5 min before analysis was considered evidence of noncompliance with the request for prompt notification; motility data for only one sample from one volunteer during the course of the study were deleted because of noncompliance.

*Ejaculate volume and frequency*

Compared with when they were consuming 10.4 mg Zn/d, volunteers consuming 1.4 mg Zn/d exhibited lower semen volumes (**Table 4**). Regression analysis of individual semen volume vs duration of experiment (regardless of zinc treatment) did not reveal any time effect on semen volume (data not shown) except for two volunteers who exhibited decreased semen volume over time ($r$ = 0.48, 0.46; $P < 0.05$). Furthermore, the number of ejaculations (voluntary + involuntary) did not differ between any two treatments (Table 4).

The number of elapsed hours between each required collection and the most recent previous ejaculation were calculated to determine length of abstinence. Seven of the volunteers reported no change in length of abstinence between the 1.4 and 10.4 mg Zn/d treatments (57, 72, 96, 288, 288, 288, or 336 h); four volunteers reported an increased period of abstinence while consuming the zinc-deficient diet (56 vs 48; 96 vs 72; 72 vs 48; and 120 vs 56 h). The length of abstinence correlated with seminal volume in only one volunteer ($r = 0.49$; $P < 0.05$). All andrology data from that individual are included because the number of elapsed hours between the required collection and the previous ejaculation was the same in both the 1.4- and 10.4-mg Zn/d dietary periods. The largest number of ejaculations per dietary period was consistently contributed by the same volunteer throughout the study. On the other hand, four volunteers reported ejaculations only at times that corresponded with the obligatory three collections per dietary period.

*Seminal mineral content*

Compared with when they were consuming 10.4 mg Zn/d, volunteers consuming 1.4 mg Zn/d had elevated concentrations of semen iron and also exhibited a slight tendency for elevated concentrations of semen magnesium (**Table 5**). Semen phosphorus concentrations were elevated during all phases of zinc depletion. Serum sodium concentrations were elevated during certain phases (2.5 or 4.4 mg Zn/d) of zinc depletion. The semen concentrations of calcium, potassium, and zinc were not affected by the dietary treatment. Of the minerals analyzed, sodium was found to be the most abundant element in human semen, followed in descending order by phosphorus, potassium, calcium, zinc, magnesium, and iron.

Reduced consumption of zinc (1.4, 2.5, or 3.4 mg Zn/d) decreased the total semen zinc loss per ejaculate. Total mineral loss per ejaculation also differed between the 1.4- (but not 2.5, 3.4, or 4.4) and 10.4-mg Zn/d dietary treatments for certain elements. Thus, compared with the 10.4-mg Zn/d treatment, the 1.4-mg Zn/d treatment decreased total semen calcium [20.9 vs 14.6 μmol/ejaculate; $P < 0.007$; root mean square error (RMSE) = 200]. Total semen sodium was affected similarly: 78.3 vs 57.3 μmol/ejaculate; $P < 0.05$; RMSE = 25.8. Compared with the 10.4-mg treatment, the 1.4 mg Zn/d treatment only tended to decrease total semen magnesium (11.9 vs 9.17 μmol/

TABLE 4
Effects of short-term dietary zinc depletion on ejaculation frequency, seminal volume, sperm density, motility, morphology, and serum testosterone concentrations in young men*

| | | | | | Sperm | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Head | | | | |
| Treatment† | Ejaculation frequency | Seminal volume‡ | Sperm density | Sperm motility§ | Area | Length/ breadth | Roundness‖ | Acrosome area | Serum testosterone |
| *mg Zn/d* | *events/d* | *mL/event* | *$10^9$/L* | *%* | *$\mu m^2$* | | | *$\mu m^2$* | *nmol/L* |
| 1.4 | 0.34 (0.09–0.86)¶ | 2.24**†† | 155 | 51 | 10.7 | 1.62 | 0.97 | 4.46 | 21.9‡‡ |
| 2.5 | 0.37 (0.09–0.80) | 2.75 | 125 | 37 | 10.9 | 1.61 | 0.97 | 4.20 | 26.2 |
| 3.4 | 0.35 (0.09–0.69) | 2.98 | 134 | 42 | 11.6 | 1.65 | 0.97 | 4.40 | 24.3 |
| 4.4 | 0.34 (0.09–0.69) | 2.96 | 120 | 48 | 11.3 | 1.62 | 0.96 | 3.99 | 23.2§§ |
| 10.4 | 0.35 (0.09–0.79) | 3.30 | 130 | 36 | 10.4 | 1.65 | 0.95 | 4.30 | 26.9 |
| *P* values (ANOVA) | | | | | | | | | |
| Zinc | NS‖‖ | 0.03 | NS | NS‖‖ | NS | NS | NS | NS | 0.03 |
| RMSE¶¶ | | 0.76 | 48 | | 1.3 | 0.07 | 0.02 | 0.44 | 4.0 |

* Eleven men aged 28.1 ± 5.0 y living on a metabolic ward for 208 d were studied. Initial body weight was maintained (±2%) by prescription exercise and adjustments in energy intake. The basal diet was composed of a mixture of conventional foods low in zinc and a synthetic formula.

† Average daily zinc intake as calculated from the analysis of 3-d composite basal diets. Each daily basal diet was supplemented with 0, 1, 2, 3, or 9 mg Zn as $ZnSO_4$. After an equilibration period of 28 d (10.4 mg Zn/d), all treatments were presented for 35 d each, the first four in random order and the 10.4-mg/d treatment last.

‡ Semen samples were collected in nonlubricated, nonspermicidal latex condoms at the end of each dietary period. Volunteers were requested to refrain from voluntary ejaculation for ≥ 48 h before collection.

§ Percent vigorously swimming 30 min postejaculation.

‖ Convex perimeter/perimeter.

¶ $\bar{x}$ (range).

** Least-squares mean for all variables except for ejaculation frequency and sperm motility.

††‡‡§§ Significantly different from 10.4 mg Zn/d (repeated-measures ANOVA): ††$P < 0.002$; ‡‡$P < 0.005$; §§$P < 0.05$.

‖‖ Ejaculation frequency and sperm motility analyzed by Friedman Test; Chi-square = 5.05.

¶¶ Root mean square error.

ejaculate; $P < 0.06$; RMSE = 76.5). On the other hand, compared with the 10.4-mg treatment, the 1.4-mg Zn/d treatment increased total semen iron (0.07 vs 0.12 μmol/ejaculate; $P < 0.04$; RMSE = 2.57).

The total amount of zinc lost per day [total seminal zinc in last collection in dietary periods × (average number of ejaculations/period)] was marginally affected by the overall dietary zinc treatments but did not change significantly between any of the zinc-depletion dietary treatments and the zinc-adequate dietary treatment. The dietary treatments did not affect the total daily loss of any other analyzed element. When the volunteers were consuming the zinc-adequate diet, the total daily loss (μmol) of each analyzed element as follows ($\bar{x} \pm$ SD): sodium, 136 ± 92; potassium, 33.7 ± 24.3; phosphorus, 33.3 ± 25.2; calcium, 7.83 ± 5.9; zinc, 2.14 ± 1.58; magnesium, 4.20 ± 3.08; and iron, 0.02 ± 0.01.

*Sperm density, motility, and morphology*

Volunteers consuming 1.4 mg Zn/d had a tendency for higher sperm densities than when they were consuming 10.4 mg Zn/d (Table 4). Regardless of dietary treatment, a time effect increased sperm density in three volunteers ($r = 0.51$, 0.57, and 0.68; $P < 0.05$) and decreased density in one volunteer ($r = 0.82$; $P < 0.01$).

Sperm motility decreased an average of 28% between 5 and 30 min after collection and was not significantly affected by the dietary treatments (Table 4). However, a treatment of 1.4 mg, compared with 10.4 mg Zn/d, tended to increase sperm motility. Regardless of dietary treatment, an increase in either semen calcium or potassium concentration decreased sperm motility 30 min postejaculation (calcium: $r = -0.32$, $P < 0.0001$; potassium: $r = -0.22$, $P < 0.008$). There was no correlation between sperm motility and semen magnesium or sodium concentrations.

Compared with the 10.4-mg Zn/d treatment, the low-zinc treatments induced a slight tendency towards decreased spermatozoa roundness (convex perimeter/perimeter) (Table 4). The low zinc treatments did not affect sperm-head area or aspect ratio (length/breadth) or acrosome area. The CVs for those variables were within acceptable limits (12%, 12%, and 4%, respectively).

*Andrology biochemistry and indices of zinc status*

The dietary treatment did not affect sperm acrosin activity (not shown) but did affect testosterone. Compared with when they were consuming 10.4 mg Zn/d, volunteers consuming either 1.4 or 4.4 mg Zn/d exhibited decreased serum testosterone concentrations (Table 4). When the volunteers consumed 1.4 mg (but not 2.5, 3.4, or 4.4) Zn/d, plasma zinc concentrations decreased (Table 5) and overall zinc balance became negative (data not shown).

TABLE 5
Effects of short-term dietary zinc depletion on semen mineral element concentrations and seminal zinc loss in young men*

| | Mineral concentration | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Semen‡ | | | | | | | Plasma | Total ejaculatory zinc loss | |
| Treatment† | Na | P | K | Ca | Mg | Zn | Fe | Zn | | |
| *mg Zn/d* | *mmol/L* | *mmol/L* | *mmol/L* | *mmol/L* | *mmol/L* | *mmol/L* | *μmol/L* | *μmol/L* | *μmol/event* | *μmol/d* |
| 1.4 | 102§ | 32.9‖ | 25.5 | 6.61 | 4.20 | 1.71 | 54.6‖ | 11.2‖ | 3.81‖ | 1.19 |
| 2.5 | 105¶ | 31.0¶ | 24.4 | 6.16 | 3.42 | 1.59 | 24.9 | 13.6 | 4.68** | 1.81 |
| 3.4 | 102 | 34.2†† | 25.0 | 6.46 | 3.70 | 1.73 | 37.8 | 14.4 | 5.03‡‡ | 1.59 |
| 4.4 | 113§§ | 33.6‖ | 27.5‖‖ | 6.54 | 3.74 | 1.88 | 32.4 | 14.7 | 5.43 | 1.82 |
| 10.4 | 98 | 28.4 | 23.6 | 6.41 | 3.54 | 1.77 | 24.0 | 13.8 | 6.29 | 2.14 |
| *P* value (ANOVA) | | | | | | | | | | |
| Zinc | 0.006 | 0.0004 | NS | NS | NS | NS | 0.009 | 0.0001 | 0.01 | 0.06 |
| RMSE¶¶ | 7 | 2.9 | 2.8 | 0.85 | 0.78 | 0.41 | 19.8 | 1.5 | 1.49 | 0.72 |

* Eleven men between the ages of 21 and 39 living on a metabolic ward for 208 d were studied. Initial body weight was maintained (±2%) by prescription exercise and adjustments in energy intake. The basal diet was composed of a mixture of conventional foods low in zinc and a synthetic formula.

† Average daily zinc intake as calculated from the analysis of 3-d composite basal diets. Each daily basal diet was supplemented with 0, 1, 2, 3, or 9 mg Zn as $ZnSO_4$. After an equilibration period of 28 d (10.4 mg Zn/d), all treatments were presented for 35 d each, the first four in random order and the 10.4-mg/d treatment last.

‡ Semen samples were collected in nonlubricated, nonspermicidal latex condoms at the end of each dietary period. Volunteers were requested to refrain from voluntary ejaculation for ≥ 48 h before collection.

§ Least-squares mean.

‖¶**††‡‡§§‖‖ Significantly different from 10.4 mg Zn/d (repeated-measures ANOVA): ‖$P < 0.001$, ¶$P < 0.05$, **$P < 0.02$, ††$P < 0.0001$, ‡‡$P < 0.06$, §§$P < 0.0003$, ‖‖$P < 0.01$.

¶¶ Root mean square error.

## Discussion

*Ejaculate volume and frequency*

The change in semen volume is probably an effect of the dietary treatment, and not of time, because semen volume did not increase with time, and, in fact, decreased with time in one volunteer. The change in volume is not related to abstinence because length of abstinence before the required collection remained constant for seven of the volunteers (minimum of 48 h as requested) and actually increased in the other four subjects during the zinc-deficient phase of the experiment.

Two previous studies allude to changes in semen volume during zinc depletion. Abbasi et al (24) reported the average spermatozoa concentration and total sperm count in five volunteers fed various amounts of zinc for various lengths of time during equilibration, depletion, and repletion. The average semen volumes (calculations based on single means) for those time periods were 3.64, 3.39, 0.91 (early repletion), and 2.01 mL (late repletion), respectively. Baer and King (25) reported average zinc concentration and total zinc/ejaculate from six volunteers fed 15.7 mg Zn/d during a short equilibration period (1 wk), then 0.28 mg/d until plasma zinc fell below 70 μg/100 g (4–9 wk). The average semen weights (based on single means) calculated for those time periods were 2.52 and 3.13 g, respectively. Thus, semen volume during zinc deficiency may have decreased in one study and tended to increase in the other. It is difficult to make comparisons between the previous studies and the present study because of the uncontrolled lengths of equilibration, depletion, and/or repletion in the early studies.

There are several factors that are known to enhance semen volume. Tulandi and McInnes (26) provide evidence that seminal volume is at least partially controlled by a hypothalamo-pituitary accessory gland axis. Other studies reported a positive correlation between the length of short-term abstinence and seminal volume. For example, Sauer et al (27) reported increased volume measured over 12, 24, 72, and 120 h of abstinence ($P < 0.0001$), but only two time periods differed significantly (12 and 120 h; $P < 0.0006$). However, a minimum of 48 h abstinence seems to be sufficient recovery time because further abstinence did not affect volume in 10 of 11 volunteers in the present study. Oligospermic or azoospermic men exhibit increased volume when treated with drugs such as bunazosin ($\alpha$ 1-blocker) or procaterol ($\beta$-stimulator) (28). Contrary to the findings of the present study (data collected from January through June), Reinberg et al (29) reported high amplitude seasonal variation with maximal seminal volumes for the year occurring in April to May. Poland et al (30) noted great stability in the volume of semen samples from normal subjects collected every 2 wk over a 6-mo period.

There are also several factors known to decrease semen volume. Compared with nonsmoking control subjects, persons who smoked > 16 cigarettes/d (31) exhibited decreased seminal volumes. Nagy et al (32) reported that, compared with nonalcoholics of known fecundity, alcoholics exhibited decreased seminal volumes. Although Shrivastav et al (33) reported that seminal volume is resistant to insulin-dependent diabetes mellitus (IDDM), the findings of Handelsman et al (34) indicate that IDDM reduces seminal volume. Other causes of decreased seminal volumes

include hyperprolactinemia (35), primary hypothyroidism (36), and leukospermia (37).

A model to explain the mechanism by which body zinc is partially conserved by reductions in seminal volumes, and not seminal zinc concentrations, relies on previous findings that suggest that *1*) citrate is the predominant anion in prostatic fluid, *2*) citrate complexes with calcium, magnesium, and zinc (38), *3*) prostatic fluid citrate concentrations are more than double the total divalent metal (including zinc) concentrations (39), and *4*) prostatic fluid citrate secretion is under androgenic control at the level of the membrane-bound Na/K ATP-dependent pump (15). The ionic charge transferred by the secretion of citrate, which is an actively secreted ion with low membrane permeability, causes transmembrane movement of potassium, chloride, and (to a lesser extent) sodium (39). Because shifts of those ions do not completely satisfy the new equilibrium conditions, the remaining negative charge induced by citrate is neutralized by the cotransport of calcium, magnesium, zinc, undissociated hydrogen, and nondiffusable cations (probably polyamines such as spermine) (39). According to osmotic pressure equations, the prostatic secretion of organic and inorganic particles would drive the diffusion of water from the prostatic parenchyma into the lumen of the gland. Thus, zinc deficiency, with a direct influence on Leydig cell output (40), could ultimately decrease prostatic fluid production through the intermediate influence of androgens on prostatic citrate secretion (15). In the present study, zinc depletion (1.4 mg/d), compared with zinc adequacy (10.4 mg/d), decreased both serum testosterone concentrations and seminal volumes.

The seminal vesicles contribute 60% to 80% of the seminal volume and secrete considerable amounts of fructose into the seminal fluid (41). There is also an association between serum testosterone concentrations and seminal corrected fructose concentrations (42). It seems reasonable that seminal vesicle secretion may also be affected indirectly by zinc deficiency, a condition that decreases testosterone production. In any event, one of the first signs of zinc depletion may be reduced seminal volume, induced by changes in Leydig cell output. The overall mean ejaculate volume was within the range reported elsewhere for normal subjects (30, 43).

Correct estimates of ejaculatory frequency are critical for zinc-balance data because Baer and King (25) found that, in two of their volunteers, the content of zinc in one seminal emission equalled or exceeded the total of all other daily zinc losses (feces, urine, integument, hair, and nails). The average number of ejaculations per day reported in the present study (0.35) is much lower than that estimated by Baer and King (one per day).

### *Mineral metabolism*

*Zinc.* The data suggest that calculated zinc balance for zinc-deprived males will be incorrect if seminal zinc loss is disregarded. In other words, 9% of the daily zinc intake can be lost via ejaculated semen when zinc intake is low (1.4 mg/d). The actual percentage may be even higher than that suggested by the experimental model; Purvis et al (44) found that volume was greater in individuals who collected samples during coitus (4.99 mL) instead of masturbation (3.93 mL). In any event, body-zinc stores were partially conserved by a decrease in the rate of zinc-rich secretions from accessory sex glands. There were no differences in the number of ejaculations or changes in seminal zinc concentrations between any two dietary treatments. The percent zinc lost via semen becomes negligible at normal dietary zinc intakes.

Despite the differences in experimental protocols, the findings from the present study and from that of Baer and King (25) suggest that zinc-conservation mechanisms are activated in the male reproductive tract during states of zinc deficiency. In both studies, the total amount of zinc per ejaculum declined significantly during depletion. There seems to be at least two zinc-conservation mechanisms, which differ according to the severity and/or length of the deficiency. Thus, body stores of zinc are partially conserved in the male by *1*) a large decrease in seminal zinc concentration (and possibly a concurrent marginal increase in volume) (25) with a resultant decrease in overall seminal zinc loss or *2*) a decrease in seminal volume with no change in concentration with a resultant decrease in overall seminal zinc loss.

Although the secretions of the seminal vesicles account for a large part of the total semen volume (45), seminal zinc is thought to be derived almost exclusively from prostatic secretions (46). Because of the affinity of citrate for zinc, calcium, and magnesium, Kavanagh (39) postulated that those metals are secreted in the prostate in an energy-independent manner, each related linearly to secreted citrate concentrations. Thus, because not all citric acid is associated with metals (including zinc) (39), it seems reasonable that zinc secretion may remain constant even as citrate secretion begins to fall (as proposed in zinc deficiency), provided that the supply of prostatic parenchymal zinc remains constant. Further depletion in body-zinc stores (including the prostate) could result in decreased seminal zinc concentrations. The mean zinc concentration in ejaculates of volunteers consuming the zinc-adequate diet was in the range reported elsewhere for normal subjects.

*Phosphorus.* The elevation in semen phosphorus concentrations induced by all phases of zinc depletion (1.4, 2.5, 3.4, or 4.4 mg/d) is noteworthy. Because secretions of the seminal vesicles are the major source of phosphorus in human semen (47), the findings suggest that zinc deprivation affects seminal vesicle function. The mean ejaculate phosphorus concentration of volunteers consuming the zinc-adequate diet was similar to that reported elsewhere for normal subjects (47).

*Potassium.* The finding that semen potassium concentrations correlate negatively with spermatozoa motility corroborates previous findings. The sodium-potassium ratio or potassium concentration per se in semen may be an important factor in sperm motility [*see* references in Quinn et al (48)]. The high concentration of potassium ion in the male reproductive tract may be responsible for the immotility of epididymal sperm (49).

*Calcium.* Although the zinc treatments did not affect semen calcium concentrations, motility decreased with increased semen calcium concentrations. Calcium is thought to regulate the initiation and maintenance of motility (50) and calcium chelators stimulate sperm motility in ejaculated human semen (51). Motility and viability were also enhanced in semen samples containing less than the average concentration of ionized calcium compared with samples with higher $Ca^{++}$ concentrations (52). Tash and Means (53) found sperm motility to be inhibited by elevated intracellular calcium. On the other hand, Prien et al (54) found a decrease in seminal $Ca^{2+}$ in those men exhibiting decreased motility, as compared with men with normal sperm motility. The mean calcium concentration in ejaculates of volunteers consuming the zinc-adequate diet was in the range reported elsewhere for normal subjects.

*Magnesium.* The magnesium in seminal fluid originates mainly from the prostate (55). Stegmayr and Ronquist (56) found that the progressive motility of washed sperm was greatly stimulated or inhibited by 2–8 or 8–16 mmol mg/L, respectively. This finding was not confirmed in the present study; there was no correlation between semen magnesium concentration (range 1.1–8.5 mmol/L) and sperm motility. The mean magnesium concentration in ejaculates of volunteers consuming the zinc-adequate diet was in the range of several values reported elsewhere for normal subjects.

*Iron.* The iron data collected in the present study were highly variable (CV 62%) and the mean ejaculate iron concentration for volunteers consuming the zinc-adequate diet was higher than that reported elsewhere for normal subjects, 5.21 ± 1.31 (57) and 8.49 ± 0.56 μmol/L (58). However, Harris et al (59) reported a seminal iron concentration of 22.6 ± 5.19 μmol/L in previously infertile men after 60 d of ascorbic acid medication.

*Sperm density, motility, and morphology*

The findings of the present study suggest that sperm density is not a good indicator of short-term zinc depletion. Abbasi et al (24) reported that dietary zinc affected mean sperm density in volunteers fed diets that contained different concentrations of zinc for variable lengths of time (all longer than those in the present study): equilibration, 305.9; zinc restriction, 254; early phase zinc repletion, 59.2; and late phase zinc repletion, 207.0 $\times 10^9$/L. Oral zinc sulfate treatment tended to decrease sperm density (29.3–26.5 $\times 10^9$/L) in 40% of patients with idiopathic asthenozoospermia and/or oligozoospermia (9) but increased density in other oligospermic men (60, 61).

Although there were negative correlations between motility and calcium or potassium concentrations in the present study, the dietary zinc treatment per se did not affect motility. This implies that the zinc pool required for spermatozoa motility is not compromised in short-term zinc depletion. Behne et al (13) found no correlation between the contents of zinc in seminal plasma or whole semen and sperm motility in 176 men with suspected infertility. Another laboratory reported increased sperm motility in infertile males treated with zinc sulfate in one study (62) but not in a second (40).

This study represents the first attempt to quantitatively assess microscopic changes induced by zinc depletion in human spermatozoa morphology. By visual microscopic analysis, Behne et al (13) found no correlation between the contents of zinc in seminal plasma or whole semen and normal morphology. As measured by image analysis, the tendency for a slight increase in sperm-head roundness induced by zinc depletion in the present study may be important. The tendency for increased roundness probably is not caused by osmotic swelling because seminal fluid is hypertonic (63) and the rise in seminal phosphorus concentrations during zinc depletion should further increase this tonicity.

Katz et al (64) found that, compared with those of fertile men, spermatozoa of infertile men had an increased length-width ratio and tended to have a decreased area (length × width). The sperm length-width ratio and area were not changed by the dietary treatment in the present study.

In summary, the findings suggest that serum testosterone concentrations, seminal volume, and total seminal zinc loss per ejaculation are responsive to short-term zinc depletion in young men.

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